STAT1-deficient T cells were indeed deficient in Th1 cells post-BMT. Relative to recipients of WT T cells, recipients of STAT1-KO T cells had reduced capacity to secrete IFN-γ (4661±664 vs. 2656±281; $p < 0.03$) and TNF-α (3154±269 vs. 1872±84; $p < 0.001$) and reduced absolute numbers of splenic CD4+IFN-γ+ T cells (14±1.9 vs .07±.02; $p < 0.05$) and CD8+IFN-γ+ T cells (4.1±1.2 vs. 0.8±.1; $p < 0.05$). As such, recipients of STAT1-deficient T cells were indeed deficient in Th1-polarization post-BMT during cGVHD. Remarkably, relative to WT T cell recipients, recipients of STAT-1 deficient T cells had increased absolute numbers of post-BMT splenic CD4+IL-17± T cells (0.2±0.05 vs 13.2±6.5.; $p < 0.05$). These data indicate that long-term post-BMT deficiency of Th1-type cells does not protect against chronic GVHD, perhaps in part due to expansion of alternative pathogenic T cells such as the Th17 subset.

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THE ROLE OF COMPLEMENT SYSTEM IN THE PATHOGENESIS OF GRAFT VERSUS HOST DISEASE

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Introduction: Graft-versus-host disease (GVHD) remains a major cause of morbidity and mortality in bone marrow transplant (BMT) recipients. GVHD is classically described as T-cell lymphocytic infiltration followed by destruction of tissue caused by differences in HLA. However, treatments aimed at other components of the immune system have been successful, questioning this paradigm. The role of the complement system in GVHD is limited. In solid tumor transplantation, activation of the complement system and link to preformed anti-HLA antibodies in recipients has been associated with rejection. We describe the evaluation of complement activation in 53 patients with clinical GVHD in whom 40 specimens of skin and 7/13 specimens of colon showed evidence of GVHD by conventional histochemical staining method. In addition, we analyzed 11 control patients with normal colon biopsies. Analysis of complement fixation was performed using C4d antibody staining method and was analyzed using the Banff07 grading system where minimal staining is denoted as positive in < 10% of vessels and diffuse staining is positive in > 50% of vessels. Histology of GVHD was correlated with C4d deposition. Statistical analysis using Fisher's exact test was performed between groups and with controls.

Results: Thirty-four of forty skin biopsies were evaluable for C4d deposition (6 specimens showing extensive background deposition were excluded). Twenty-one of thirty-four specimens showed C4d staining (11 minimal, 9 focal, and 1 diffuse). Twelve of the thirteen colon biopsies showed C4d staining (2 minimal, 4 focal, and 6 diffuse). Of the 11 control colon biopsies, 10 were negative for C4d and 1 showed minimal staining. In the cohort that showed clinical GVHD not pathologically confirmed, 6/6 showed C4d staining. The difference in C4d staining between the GVHD of the colon and colon control specimens was statistically significant using a Fisher's exact test (p-value = 0.00000177). In addition, there was statistical difference between Cd4 staining in clinical GVHD vs. controls with a p value of 0.0000127.

Conclusion: Our results demonstrate that most patients with clinical GVHD showed vascular staining for C4d. Clinical GVHD and C4d deposition were closely correlated and statistically different from controls. This may indicate that C4d positivity is a more sensitive marker in detecting GVHD of the colon than classic GVHD histological evaluation.

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SUSTAINED HIGH-DOSE CORTICOSTEROID USE DOES NOT INDEPENDENTLY DIMINISH SURVIVAL AFTER MYELOABLATIVE ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Introduction: Corticosteroids remain the most efficacious treatment for acute GVHD (aGVHD) following allogeneic hematopoietic stem cell transplantation (AlloHSCT). Some patients with aGVHD receive high-dose (HD) corticosteroids for ≥ 2 months for a variety of reasons. Morbidities related to long term corticosteroid use are well recognized, however, the impact of dosing and treatment duration on survival has not been fully evaluated in the post-AlloHSCT setting.

Methods: From 1998 to 2009, 272 patients with AlloHSCT were treated with corticosteroids between post-transplant days 30 and 100 at a single academic institution. Patients in HD group received ≥ 30 mg prednisone daily for ≥ 2 months, whereas those in the low-dose (LD) corticosteroid group received < 30 mg at some point in this interval or ≥ 30 mg for < 2 months. Relapse-free (RFS) and overall survival (OS) were compared between corticosteroid groups. Cox proportional hazards analysis identified prognostic factors.

Results: HD (n = 98) and LD (n = 174) groups were similar in distribution of demographic characteristics, comorbidity index, diagnosis, number of previous chemotherapies and conditioning regimen (all $p \geq 0.3$). HD group had more MUD AlloHSCT ($p = 0.05$) and higher-grade aGVHD ($p < 0.001$). Malignancy relapse in HD and LD groups was 24% and 36%, whereas overall mortality was 59% and 55% respectively. Non-relapse mortality tended to be higher (HR = 1.5; 95% CI, 1.0-2.3) in HD group. No significant association was detected between the HD corticosteroid use and infection-related mortality ($p = 0.1$), relapse mortality ($p = 0.2$), RFS ($p = 0.8$), or OS ($p = 0.4$). Multivariable analysis of non-relapse mortality demonstrated no independent prognostic significance of HD corticosteroid use (HR = 1.3; 95% CI, 0.8-2) after adjustment for comorbidity index (HR = 2 for high vs. low HCT-CI; 95% CI, 0.8-2), source of hematopoietic stem cells (HR = 2.3 for peripheral vs. bone marrow; 95% CI, 1.3-4.1) and grade of aGVHD (HR = 1.3 per 1 grade increase; 95% CI, 1.1-1.6).

Conclusions: Using HD corticosteroids for ≥ 2 months did not appear to have an independent impact on patient survival following AlloHSCT and therefore it may be safe to use as necessary for aGVHD control. The association of HD steroids with non relapse mortality appears to be related to worse aGVHD.

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THYMIC IRRADIATION IS REQUIRED FOR TRANSPLANTATION TOLERANCE AFTER TLI/ATS NON-MYELOABLATIVE CONDITIONING

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Total Lymphoid Irradiation and Anti-Thymocyte Serum (TLI/ATS) conditioning induces potent donor-host tolerance and GVHD protection via induction of donor $CD4^+CD25^+Foxp3^+$ Treg after bone marrow transplantation (BMT) (Pillai et al, Blood 2009). Whereas total body irradiation (TBI) at doses as low as 400 cGy associate with lethal acute GVHD after MHC-mismatched BMT, TLI exposes GVHD target organs to a cumulative radiation dose of 2400 to 4080 cGy, without GVHD. We investigated the role of radiation exposure to specific anatomic areas in tolerance induction after TLI/ATS + BMT. Wild-type (WT) BALB/c ($H\text{-}2^d$) hosts received infusion of 50 x 10^6 bone marrow + 60 x 10^6 splenocytes from WT C57BL/6 ($H\text{-}2^b$) donors (BMT) following 5 doses of rabbit ATS + 10 doses (240 cGy each2400 cGy total) of fractionated TBI, TLI, or TLI with lead shielding of specific regions, including TLI with focal thymic shielding (TLI-FTS). Day 6 $H\text{-}2K^{b+}CD8^+$ and $H\text{-}2K^{b+}CD4^+CD25^{neg}$ effector cell and $H\text{-}2K^{b+}CD4^+Foxp3^+$ Treg accumulation was quantified in target organs, and GVHD scoring performed on histopathologic sections. Positive controls included WT BALB/c hosts receiving ATS + single-dose 800cGy TBI (PC-1) or ATS + 400 cGy TBI (PC-2) and 50 x 10^6 bone marrow + 60 x 10^6 splenocytes from C57BL/6 donors. Negative controls received 800cGy TBI and 50 x 10^6 bone marrow from WT C57BL/6 (NC-1) or ATS alone + BMT (NC-2). All fractionated TBI hosts died during conditioning with histopathologic systemic radiation toxicity. In TLI/ATS hosts at day 6 after BMT, accumulation of $H\text{-}2K^{b+}TCR^+CD8^+$ cells was strongly suppressed in colon, MLN, and spleen ($p < 0.01$), with increased accumulation of donor Treg ($p < 0.01$) compared to PC-1 controls. Mean colonic

GVHD scores were: PC-1 (n = 5): 6 ±0.7; NC-1 (n = 4): 0; NC-2 (n = 4): 0; TLI/ATS (n = 4): 0.4±0.8; TLI-FTS/ATS (n = 7): 4.5±0.9, with similar patterns in liver. Day 6 target organ accumulation of H-2K^{b+}CD4^{+}Foxp3^{+} Treg was markedly decreased ($p < 0.05$) and H-2K^{b+}CD8^{+} cells increased ($p < 0.01$) in TLI-FTS/ATS hosts. In conclusion, shielding from thymic irradiation alone ablates tolerance after TLI/ATS + BMT, indicating that recurrent thymic radiation exposure in the non-myeloablative setting enhances rather than impedes early immune mechanisms leading to transplantation tolerance in this regimen. We are currently delineating novel thymic pathways of enhanced immunoregulatory cell selection in thymic-irradiation-induced transplant tolerance after TLI/ATS + BMT.

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HOST MYELOID-DERIVED SUPPRESSOR CELLS INDUCE DONOR TREG PROLIFERATION AND TRANSPLANTATION TOLERANCE VIA IL-4Rα/STAT6 AFTER TLI/ATS NON-MYELOABLATIVE BMT

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Murine total Lymphoid Irradiation and rabbit Anti-Thymocyte Serum (TLI/ATS) non-myeloablative conditioning has been translated for tolerance induction to facilitate engraftment without lethal GVHD after allogeneic HSCT. Tolerance is mediated by host IL-4-induced expansion of donor CD4^{+}Foxp3^{+} Treg from the unmanipulated graft (Pillai et al, Blood 2009). To delineate mechanisms of IL-4-dependent donor Treg expansion, wild-type (WT) BALB/c (H-2^{d}) hosts were given 3 doses ATS + 17 doses (240 cGy each) TLI and infusion of 50 x 10^{6} bone marrow and 60 x 10^{6} CFSE-labeled splenocytes (BMT) from WT or STAT6$^{-/-}$ C57BL/6 (H-2^{b}) donors. Control WT BALB/c were given 800 cGy single-dose total body irradiation (TBI) with either 50 x 10^{6} bone marrow alone (negative control, NC-1) or 50 x 10^{6} bone marrow + 60 x 10^{6} splenocytes (positive control, PC) from WT C56BL/6 donors. Day 6 H-2K^{b+}TCR^{+}CD8^{+} cell accumulation in spleen, liver and colon were potently inhibited, and H-2K^{b+}CD4^{+}Foxp3^{+} Treg proliferation maintained, after STAT6$^{-/-}$ donor BMT, indicating that host IL-4 effects on donor Treg proliferation is indirect. By contrast, IL-4Rα$^{-/-}$ BALB/c hosts receiving TLI/ATS+BMT developed dramatic lethal acute GVHD [day 6 colonic GVHD scores: PC (n = 5) 6 ±0.8; NC-1 (n = 5): 0; WT TLI/ATS (n = 10): 1.3 ±0.9; IL-4Rα$^{-/-}$TLI/ATS (n = 10): 5.8 ±1.3]. We delineated 2 subsets of IL-4Rα/CD124^{hi} cells [CD11b^{+}Gr-1G^{hi} (M1) and CD11b^{+}Gr-1G^{int} (M2)] that dominate the host myeloid compartment in WT hosts at both day 0 after TLI/ATS and day 6 after TLI/ATS + BMT vs TBI/ATS or TBI/ATS + BMT ($p < 0.05$). Both M1 and M2 subsets are F4/80^{neg}CD115^{neg}B220^{neg}CD11c^{neg}CD1d^{hi} myeloid derived suppressor cells (MDSC), immature marrow-derived cells which are ablated after lethal or sublethal TBI ($p < 0.05$). H-2K$^{b\text{-}neg}$ M1 and M2 populations sorted from host spleens at day 6 after TLI/ATS + BMT stimulated H-2K^{b+}CD4^{+}Foxp3^{+} Treg but not H-2K^{b+}CD4^{+}CD25^{neg}Foxp3^{neg} or H-2K^{b+}CD8^{+} effector proliferation in 72-hr CFSE MLR with C57BL/6 splenocyte responders. Day 6 H-2K$^{b\text{-}neg}$ M1 and M2 cells sorted from TLI/ATS + BMT-treated STAT6$^{-/-}$ or IL-4Rα$^{-/-}$ BALB/c hosts were unable to induce H-2K^{b+}CD4^{+}Foxp3^{+} Treg proliferation in MLR, supporting an IL-4/STAT6-inducible pathway by which these MDSC gain capacity to induce Treg proliferation. Using hosts deficient in key IL-4/STAT6-inducible proteins (Arginase, NOS-2) in MDSC, we are now delineating the MDSC pathway inducing Treg proliferation and tolerance after TLI/ATS + BMT.

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HERPES SIMPLEX VIRUS INDUCED STEM CELL DIFFERENTIATION AND ASSOCIATION WITH GRAFT-VERSUS-HOST DISEASE (GVHD) IN BONE MARROW TRANSPLANT RECIPIENTS

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Viral pathogens have been implicated in the pathogenesis of GVHD. Skin rash due to GVHD resembles herpes simplex virus (HSV)-associated erythema multiforme (EM), in which HSV-infected CD34+ stem cells are stimulated to differentiate into antigen presenting CD1a+ DCs which reside in the skin epidermis and are known as Langerhans cells (LC). This differentiation involves increased E-cadherin expression. HSV-primed LCs present HSV protein to T-cells and lead to EM.

In this prospective study, we examined (1) HSV-induced CD34+ cell differentiation and (2) Expression of the HSV Pol gene in blood MNC and tissue samples of allogeneic SCT patients obtained before and after the transplant. HSV prophylaxis was oral acyclovir. Second skin biopsy was obtained only from patients who developed a rash posttransplant. Double immunofluorescent staining and FACS analysis were used to quantify the expression of the HSV antigen (Pol), CD34 and differentiation-associated markers (e.g., E-cadherin).

A total of 35 patients were enrolled. Data are available in 27 patients. Twenty-two (81%) had negative pol, CD34/pol, CD14/pol, CD11b/pol, CD1a/pol immunostaining in the skin at baseline. Group I: Twelve patients, 7 male, developed GVHD. In this group, 11 (92%) patients became pol+ and 9 had CD34+/pol+ in the skin. Nine out of 11 (82%) patients had increased number of CD34+/Pol+ expressing PBMNCs. Seven out of 11 (64%) and 6 out of 7 (86%) patients had increased CD34+/E-cad+ and CD1a+/Pol+ PBMNCs, respectively. Group II: Six patients (22%), all male, developed rash but no proven GVHD. Only 2 out of 4 patients (50%) with non-GVHD rash were pol+ in the skin post-SCT. Level of antigen expression in this group all decreased post-SCT blood samples. Group III: Eleven patients, 7 of them female, had no rash post-SCT and all of them remained pol-negative in blood samples post-transplant. Four patients (36%) had slight increase in CD34+/Pol+ expressing PBMNCs compared to baseline. CD34+/E-cad+ cells were noted to be minimally increased in 2 patients (18%) only.

In conclusion, our data suggest that HSV can be reactivated at the cellular level after allogeneic SCT and appears be associated with the occurrence of skin GVHD. Differentiated LCs in the skin following HSV infection of the circulating CD34+ cells may well be part of this association. Additional studies are warranted to confirm these results and consider new strategies to prevent GVHD, including perhaps more intense HSV prophylaxis.

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DOUBLE HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) RESULTS IN SUCCESSFUL ENGRAFTMENT OF BONE MARROW FROM BOTH DONORS WITHOUT GRAFT VERSUS HOST OR GRAFT VERSUS GRAFT EFFECTS

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We hypothesize that the use of two haploidentical donors, each targeting a different recipient haplotype, will increase anti- leukemia activity after double haploidentical SCT. We first established a haploidentical transplant model using two different hybrid mouse strains as donor and recipient in the experiments. Lethally irradiated B6CBAF1 (H2Kb/k) recipients were transplanted with T cell depleted (TCD) bone marrow (BM) from B6D2F1 (H2K$^{b/d}$) donors. Recipient mice harvested at days 28, 42 and 84, showed more than 90% donor cell engraftment, including donor derived lymphopoiesis and myelopoiesis, without evidence of graft versus host disease. Subsequently, lethally irradiated B6CBAF1 (H2K$^{b/k}$) recipients were transplanted with TCD-BM from two haploidentical donors (DH model) including B6SJLF1 (H2K$^{b/s}$) (donor 1 - D1) and B6D2F1 (H2K$^{b/d}$) (donor 2 - D2). We observed recipients for 90 days and all mice survived without evidence of GVHD or weight loss. Analyses of blood collected retro-orbitally at day 90 revealed that recipients of DH transplants had significantly higher WBC and neutrophil counts than recipients of SH HSCT from either D1 or D2 respectively. DH recipients consistently showed successful engraftment with mixed chimerism in both bone marrow and spleen. We then explored the effects of low dose T cell infusions ($1x10^{5}$) on chimerism of donor cells. Low dose T cell infusion from either D1 or D2 did not affect the BM cellularity, but did increase the degree of dominance of that donor's cells in the BM and spleen.

Recipients of TCD DH transplants were challenged with P815 tumor cells. We used B6SJLF1 (D1) + B6CBAF1 (D2) → B6D2F1 model in tumor experiments. Interestingly, recipients of TCD-DH